IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Michael Wassenegger et al.
Application No.	:	09/782,874 Confirmation No.: 6565
Filed	:	February 8, 2001
For	:	NUCLEIC ACID MOLECULES ENCODING POLYPEPTIDES HAVING THE ENZYMATIC ACTIVITY OF AN RNA-DIRECTED RNA POLYMERASE (RdRP)
Group Art Unit	:	1638
Examiner	:	Georgia L. Helmer

Mail Stop Petition
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF LILLIAN GARCIA

I, LILLIAN GARCIA, hereby declare that:

1. I am a Docket Clerk in the Patent Department of Fish & Neave, 1251 Avenue of the Americas, New York, New York 10020. My duties include processing and mailing documents which are to be filed in the United States Patent and Trademark Office.

2. The Fish & Neave procedure used by me and the other clerks in the Patent Department of Fish & Neave to

process a filing in the United States Patent and Trademark Office, including a Response to Office Action, comprise the following steps:

a. The filing is page-checked to ensure that: (i) each page of the submission is numbered consecutively, and (ii) each document of the submission is correctly entered on: (a) the Transmittal Letter for a Response to Office Action and (b) the return receipt postcard.

b. The Response is checked to ensure the Express Mail number printed on the first page of the Response matches the Express Mail number of the Express Mail label.

c. The Express Mail Certification is signed and placed inside an envelope with the Response and other associated documents, as listed in the Express Mail Certification.

d. The envelope is sealed, and an entry is made in an outgoing log kept in the Patent Department noting the Express Mail number, the internal docket number of the application, and the date and time of mailing.

e. The sealed envelope is then given to a clerk in the Fish & Neave Mail Room for delivery to the United States Postal Service.

f. After depositing the application with the United States Postal Service, the Mail Room clerk completes an Express Mail Order Slip noting the date, time and place of mailing.

3. In the instant case, I followed the above procedures and caused a Response to Office Action, comprising two (2) copies of a Transmittal Letter for a Response to Office Action, two (2) copies of a Petition Under 37 C.F.R. 1.136(a) for extension of time, twenty-one (21) pages of a Response to Office Action, a check in the amount of $465.00, seven (7) pages of a Declaration of Michael Wassenegger Under 37 C.F.R. 1.132, two (2) copies of a Transmittal Letter for a Supplemental Information Disclosure Statement, two (2) pages of a Supplemental Information Disclosure Statement, two (2) copies of a PTO 1449 Form, a check in the amount of $180.00, one (1) copy of an Express Mail Certification, and a return receipt postcard to be filed with the United States Patent and Trademark Office on May 20, 2003, via deposit with the United States Postal Service - Express Mail certification number EV242441619US (see first page of Transmittal Letter included in Exhibit A).

4. Attached to this declaration are true and complete copies of the documents filed with the Response to Office Action, dated May 20, 2003 (Exhibit A) - all of which

were obtained from the Fish & Neave file for the application, which is maintained by the Fish & Neave Patent Department. Attached as Exhibit B is the Fish & Neave receipt of the express mail label with the "date in" of May 20, 2003 stamped by the U.S. Postal Service. Also attached is a true copy of the acknowledgment of Receipt Card (Exhibit C), i.e., the postcard as stamped and returned to Fish & Neave by the United States Patent and Trademark Office.

5. The undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or of any patent issuing thereon.

_____
Lillian Garcia

Signed this 10th day
of September, 2003 at
New York, New York

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Georgia L. Helmer
Group	:	1638
Applicants	:	Michael Wassenegger, et al.
Serial No.	:	09/782,874
Filed	:	February 8, 2001
For POLYPEPTIDES	:	NUCLEIC ACID MOLECULES ENCODING HAVING THE ENZYMATIC ACTIVITY OF AN RNA-DIRECTED RNA POLYMERASE (RdRP)

Hon. Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MICHAEL WASSENEGGER UNDER 37 C.F.R. § 1.132

I, Michael Wassenegger, a citizen of Germany, residing at Schellingstrasse 22, 80799 Munich, Germany hereby declare that:

1. I received a diploma in biology in 1984 and a doctoral degree in biochemistry in 1988 from the University of Cologne/Max-Planck-Institute for Plant Breeding Research. I have published twenty scientific articles, and have received two patents. My curriculum vitae is attached as Exhibit 1.

2. I have studied gene silencing in plants since 1994. I have been employed by the Fraunhofer Institute for Molecular Biology and Applied Ecology since 1999 as head of the Epigenetics department.

3. I am familiar with the November 20, 2002 Office action in the above-identified application. I understand it is the Examiner's view that the claims contain subject matter which was not described in the specification in such a way as

to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention at the time the application was filed. Specifically, in the Examiner's view:

> [t]he claims are drawn to sequences that are at least 60% identical to SEQ ID NO: 1 that encodes a protein at least 60% identical to SEQ ID NO: 2 or to conservative variants thereof that have RdRP activity. However, the specification does not disclose what structural features would be conserved in the claimed sequences that would result in the claimed enzyme activity. Applicants are claiming a genus of sequences, yet there is no description of the structural features that define the genus.

See Office Action, page 7.

4. In addition, I understand that the Examiner considers the disclosure enabling only for claims directed to a nucleic acid molecule that comprises a nucleic acid sequence that is SEQ ID NO: 1, or that encodes SEQ ID NO: 2, where the nucleic acid encodes a protein that has RdRP activity. See Office Action, page 8. In the view of the Examiner, the disclosure does not provide enablement for:

> sequences that are at least 60% identical to SEQ ID NO: 1, that encodes a protein at least 60% identical to SEQ ID NO: 2 or to conservative variants thereof that have RdRP enzymatic activity . . . [or for] a nucleic acid of SEQ ID NO: 1 or a nucleic acid encoding SEQ ID NO: 2, where that nucleic acid (presence/transcription/expression) causes a reduction in synthesis of RdRP.

See Office Action, page 8.

5. I make this declaration to demonstrate that the specification adequately describes the claimed transgenic plant cells and transgenic plants comprising the nucleic acid molecule of the invention. I further make this declaration to demonstrate that sequences that are at least 80% identical to SEQ ID NO: 1 or that encode a protein at least 80% identical to SEQ ID NO: 2, as well as degenerate

sequences thereof, encode a protein having RdRP activity.[1] Finally, I make this declaration to demonstrate that one of ordinary skill in the art, following the teachings of the specification could produce transgenic plants and plant cells containing the nucleic acid molecules of the invention and would reasonably expect that such integrated constructs would, through transcription and/or expression of the nucleic acid molecule, reduce RdRP activity in the cell. Specifically, I describe experiments demonstrating the production of transgenic tobacco plants with integrated nucleic acid molecules encoding RdRP in antisense orientation that show reduced RdRP activity.

6. The specification describes a Southern blot analysis of the *Hind*III-restricted genomic DNA of two different tomato species, potato and tobacco. See Fig. 2 of the specification. The Southern blot, using a ^{32}P-labeled tomato RdRP DNA probe, reveals that the RdRP gene is present in each of the four genomes. Further, the Southern blot analysis provides evidence that the inventors had possession of a nucleic acid molecule encoding RdRP in various plant species. As discussed in ¶7 below, the nucleotide sequence comparison between the RdRP coding region of *Nicotiana tabacum* to that of SEQ ID NO: 1 shows a 89.9% sequence identity while the amino acid sequence comparison between *Nicotiana tabacum* and SEQ ID NO: 2 shows a sequence identity of 85.8%. For these reasons, I believe that the written description in the specification sufficiently conveys the structural and physical features of the claimed transgenic plant cells and plants comprising nucleic acid molecules that have at least 80% sequence identity to SEQ ID NO: 1 or that encode a protein at least 80% identical to SEQ ID NO: 2 that result in the claimed RdRP activity. Therefore, it is my belief, based on the evidence, that the specification

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[1] Three RdRP molecules have been identified since our initial identification of RdRP in plants. The originally identified RdRP molecule is now referred to as RdRP[1]. However, in this declaration, I refer to it as it was originally described.

reasonably conveys to one of ordinary skill in the art that the inventor was in possession of the claimed genus at the time the application was filed.

7. The specification enables nucleic acid molecules having at least 80% sequence identity to SEQ ID NO: 1 or to a nucleic acid molecule encoding SEQ ID NO: 2, as well as to degenerate sequences thereof. Nucleic acid molecules encoding RdRP from *Nicotiana tabacum* and *Arabidopsis thaliana* species have been isolated and sequenced. See Exhibit 2. Comparisons of these sequences to SEQ ID NO: 1 and SEQ ID NO: 2 are attached as Exhibits 3-5. The RdRP amino acid sequences of *Nicotiana tabacum* and *Arabidopsis thaliana* exhibit a sequence identity to SEQ ID NO: 2 of 85.8% and 61.9%, respectively. See Exhibit 3. The RdRP coding region of *Nicotiana tabacum* and *Arabidopsis thaliana* exhibit a nucleotide sequence identity to the coding region of SEQ ID NO: 1 of 89.9% and 63.2%. See Exhibits 4 and 5. Xie et al. (PNAS, 99:6516-6521, 2001; hereafter "Xie") demonstrates that the nucleic acid molecule encoding the *N. tabacum* RdRP (NtRdRP) has RdRP activity. See, e.g., pages 6517-6519 of Xie. Further, one having skill in the art would reasonably expect that these *Arabidopsis* nucleic acid molecules would also encode polypeptides with RdRP activity. Thus, the comparison of the nucleic acid molecules of this invention with corresponding sequences from *Nicotiana tabacum* and *Arabidopsis thaliana* demonstrate that nucleic acid molecules exhibiting a sequence identity of approximately 80% or more to the nucleic acid molecules of this invention encode a polypeptide with RdRP activity.

8. In addition, the specification teaches one of ordinary skill in the art to identify and isolate nucleic acid molecules that exhibit at least 80% sequence identity to the described nucleic acid sequences that encode a protein having RdRP activity. See page 7, line 28 to page 8, line 23 of the specification. For example, the specification also teaches and exemplifies an assay for determining whether a protein

encoded by a nucleic acid molecule of the invention has RdRP activity. See page 31, line 19-31 of the specification. Thus, given the teachings in the specification as filed, I believe that others skilled in the art could make and use nucleic acid molecules that exhibit a sequence identity of at least 80% to SEQ ID NO: 1 or that encode a protein that is at least 80% identical to SEQ ID NO: 2 without undue experimentation.

9. The specification provides enablement where the presence, expression, or transcription of a nucleic acid having at least 80% sequence identity to SEQ ID NO: 1 or a nucleic acid molecule that is at least 80% identical to a nucleic acid molecule encoding SEQ ID NO: 2 causes a reduction in RdRP activity. The specification discloses that expression of a nucleic acid molecule of the invention in a plant cell would reduce the synthesis of a polypeptide having RdRP activity due to an antisense or co-suppression effect. See page 22, lines 6-12 of the specification. The specification further teaches how to achieve such an antisense or co-suppression effect with the nucleic acids of the invention. Specifically, the specification discloses that the nucleic acid molecules of the invention are preferably linked to regulatory elements ensuring transcription in plant cells, and describes specific regulatory elements that could be used. The specification teaches that the nucleic acid molecule may be of homologous origin with respect to the plant species used for transformation or may be a heterologous nucleic acid molecule, preferably with homology of at least 80%. See page 23, lines 17-25 of the specification. The specification also teaches methods for transforming monocotyledonous and dicotyledonous plant cells with antisense nucleic acid constructs, and methods for identifying transgenic plant cells having the desired characteristics. The specification also teaches regeneration of transgenic plants from the transgenic plant cells. See page 23, line 27 to page 25, line 13 of the specification.

10. Xie discloses the suppression of inducible RdRP activity in transgenic tobacco plants into which an antisense construct of the homologous tobacco RdRP gene (NtRdRP) had been introduced. Specifically, Xie cloned a 3.8 kb NtRdRP cDNA fragment into the plant transformation vector pCK8 in antisense orientation under the regulation of the cauliflower mosaic virus (CaMV) 35S promoter. See Xie, page 6517, right column, lines 4-10. The instant specification teaches the use of expression vectors and various promoters including the 35S promoter of CaMV. See page 24, lines 3-5 and page 10, lines 4-15 of the specification. Xie introduced the NtRdRP antisense construct into tobacco by *Agrobacterium*-mediated transformation. See Xie, page 6517, right column, lines 4-10. The instant specification teaches that the foreign DNA of the invention may be introduced into plants by *Agrobacterium*-mediated transformation. See page 24, line 28 to page 25, line 2 of the specification.

11. Xie demonstrated that salicylic acid (SA) or tobacco mosaic virus (TMV) treatment induced RdRP activity in wild type plants. See Xie, Fig. 3. In contrast, transgenic tobacco plants containing the antisense RdRP construct exhibited drastically reduced RdRP activity after treatment with SA or TMV (see Xie, Figs. 3A and 3B) and contained no detectable NtRdRP transcripts after viral infection (see Xie, Figs. 3C and 3D). Xie also showed that the antisense transgenic plants accumulated higher levels of viral RNAs (see Xie, Fig. 4) and developed more severe symptoms after infection by TMV (see Fig 5) or potato virus X (see Xie, Fig 6). Thus, Xie demonstrates that expression of an RdRP nucleic acid molecule in antisense orientation reduces RdRP activity.

12. Following the teachings of the specification, I have made a transgenic tobacco plant that expressed an antisense construct comprising a nucleic acid molecule encoding a tomato RdRP. Similar to Xie, I found that transgenic

tobacco plants containing the tomato RdRP cDNA as an antisense construct exhibited drastically reduced RdRP activity after treatment with TMV and potato spindle tuber viroid (PSTVd) as shown by Northern analysis and expression of more severe symptoms upon TMV infection. Thus, the experiment demonstrates that the specification enables production of a transgenic plant comprising a heterologous antisense RdRP and that the resulting antisense construct resulted in reduced RdRP activity.

13. Furthermore, it was well known in the art at the time the invention was made that co-suppression could be used to reduce the expression of the homologous gene in plants (WO 90/12084). For this reason, I believe and reasonably expect that one of ordinary skill in the art could similarly use the nucleic acids of the invention to produce transgenic plants without undue experimentation and would reasonably expect the plants to exhibit reduced RdRP activity due to co-suppression.

14. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Michael Wassenegger

Signed this 07 day of May, 2003
at Munich, Germany

CURRICULUM VITAE

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Curriculum vitae

PERSONAL DATA

Born: 10th of August 1956 in Bonn, NRW
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EDUCATION

Studies: Biology, University of Cologne / Max-Planck-Institute for Plant Breeding Research, Cologne Vogelsang, 1977-1984, Diploma 1984

Dissertation: University of Cologne / Max-Planck-Institute for Plant Breeding Research, Cologne Vogelsang, Group of Dr. Koncz, Depart. Prof. Schell, 1984-1988

Habilitation: Rheinisch-Westfälische Technische Hochschule Aachen, 2001

EMPLOYMENT

1984 - 1988: PhD student at the MPI for Plant Breeding Research in Cologne, Group of Prof. Otten (1984), Group of Prof. Willmitzer (1985), Group of Dr. Koncz (until 1988), Depart. Prof. Schell

1988-1993: Head of team at the Max-Planck-Institute for Biochemistry in Martinsried, Depart. Prof. Sänger

1993 - 1995: Max-Planck research stipend and head of team at the Max-Planck-Institute for Biochemistry in Martinsried, Depart. Prof. Sänger

1995 - 1998: DFG habilitation stipend and head of team at the Max-Planck-Institute for Biochemistry in Martinsried, Depart. Prof. Sänger

Since 1999: Fraunhofer Institute for Molecular Biology and Applied Ecology, Head of the Epigenetic department
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MEMBERSHIPS

Society for Molecular Biology und Biochemistry (GMB)

REGULAR REVIEWING

EMBO Journal, European Journal of Biochemistry, FEBS Letters, Journal of Cell Science, Journal of Virology, Molecular & General Genetics, Plant Biotechnology Journal, Plant Molecular Biology, RNA, Theoretical Applied Genetics, The Plant Cell The Plant Journal, Transgenic Research, Trends in Plant Science, Trends in Plant Virology

Munich, 14th of April 2003

Michael Wassenegger

PUBLICATIONS

Research Publications:

Wassenegger M., Heimes S., Riedel L. and Sänger H. L. (1994). RNA-directed *de novo* methylation of genomic sequences in plants. *Cell* **76**: 567-576.

Wassenegger M., Heimes S. and Sänger H. L. (1994). An infectious viroid RNA replicon evolved from an *in vitro*-generated noninfectious viroid deletion mutant via a complementary deletion *in vivo*. *EMBO J.* **13**: 6172-6177.

Riedel L., Pütz A., Hauser M.-T., Luckinger R., Wassenegger M. and Sänger H. L. (1995). Characterization of the SRP RNA population of tomato. *Plant Mol. Biol.* **27**: 669-680.

Riedel L., Volger U., Luckinger R., Pütz A., Sänger H. L. and Wassenegger M. (1996). Molecular analysis of the gene family of the signal recognition particle (SRP) RNA of tomato. *Plant Mol. Biol.* **31**: 113-125.

Wassenegger M., Spieker R., Riedel L., Thalmeir S., Gast F.-U. and Sänger H. L. (1996). A single nucleotide substitution converts potato spindle tuber viroid (PSTVd) from a noninfectious to an infectious RNA for *Nicotiana tabacum*. *Virology* **226**: 191-197.

Schiebel W., Pélissier T., Riedel L., Thalmeir S., Schiebel R., Kempe D., Lottspeich F., Sänger H. L. and Wassenegger M. (1998). Isolation of a RNA-directed RNA polymerase-specific cDNA clone from tomato leaf-tissue mRNA. *Plant Cell* **10**: 2087-2101.

Pélissier T., Thalmeir S., Kempe D., Sänger H. L. and Wassenegger M. (1999). Heavy *de novo* methylation at symmetrical and non-symmetrical sites is a hallmark of RNA-directed DNA methylation. *Nucl. Acids Res.* **27**: 1625-1634.

Pélissier T. and Wassenegger M. (2000). A DNA target of 30 bp is sufficient for RNA-directed DNA methylation. *RNA* **6**:.55-65.

Wassenegger M. (2001). Advantages and disadvantages of using PCR techniques to characterize transgenic plants. *Mol. Biotech.* **17**: 73-82.

Bonin M., Oberstrass J., Vogt U., Wassenegger M. and Nellen W. (2001). Binding of IRE-BP to its cognate RNA sequence: SFM studies on a universal RNA backbone for the analysis of RNA-protein interaction. *Biol. Chem.* **382**: 1157–1162.

Vogt U., Pütz A., Razvi F., Pélissier T. and Wassenegger M. (2003). Viroid-mediated induction of post-transcriptional gene silencing. In preparation.

Reviews:

Wassenegger M. and Pélissier T. (1998). A model for RNA-mediated gene silencing in higher plants. *Plant Mol. Biol.* **37**: 349-362.

Wassenegger M. and Pélissier T. (1999). Signalling in gene silencing. *Trends Plant Sci.* **4**: 207-209.

Bailey-Serres J., Rochaix J.-D., Wassenegger M., and Filipowicz W. (1999). Plants, their organelles, viruses and transgenes reveal mechanisms and relevance of post-transcriptional processes. *EMBO J.* **18**: 5153-5158.

Wassenegger M. (2000). RNA-directed DNA methylation. *Plant Mol. Biol.* **43**: 203-220.

Wassenegger M. (2002a). Gene silencing. *Internat. Rev. Cytol.* **219**: 61-113.

Wassenegger M. (2002b). Gene silencing-based disease resistance. *Transgenic Res.* 11: 639-653.

Book Contributions:

Sänger H. L., Schiebel W., Riedel L., Pélissier T., and Wassenegger M. (1996). The possible links between RNA-directed DNA methylation (RdDM), sense and antisense RNA, gene silencing, symptom-induction upon microbial infections and RNA-directed RNA polymerase (RdRP). In *Biology of Plant-Microbe Interactions*, G. Stacey, B. Mullin, and P.M. Gresshoff, eds. (St. Paul, MN: American Phytopathological Society), pp. 533-540.

Wassenegger M. (1998). Application of PCR to transgenic plants. PCR in Bioanalysis. In *Methods in Mol. Biol.*, **92**, S.J. Meltzer (Humana Press Inc., Totowa), pp. 153-164.

Depicker A., De Buck S., Müller A. and Wassenegger M. (2002). Transgene expression. In Handbook of Plant Biotechnology, in press.

Patents:

US-Patent (No.: 6,218,142B1): Nucleic acid molecules encoding polypeptides having the enzymatic activity of an RNA-directed RNA polymerase (RdRP). Date of Patent: 17. 04. 2001.

EU Patent Application (No.: 01 11 9348.9): Methods and means for gene silencing in transgenic plants. Submitted 2001.

Talks:

1994 - 2002: 25/22 (Home/Abroad)